single cell analysis in GC that shows members of the same clone at different stages of mutation in the dark zone and light zone (Küppers et al., 1993). Previous 5-bromo-2′-deoxyuridine pulse chase experiments in rat GC indicate that B cells that have taken up label in the dark zone during a 3 hr labeling period appear in the light zone between 6 and 10 hr from starting labeling (Liu et al., 1991). Similar findings in mice were reported by Hanna in 1964 (Hanna, 1964). The period of postlabeling observation of Wang and Carter (2005) was too brief to pick up this phenomenon. If cells in the dark zone are to be selected it is hard to see how this could happen in situ as antigen and T cells are not obviously present. The data cited above are consistent with these cells passing into the light zone where they are selected. In germinal centers induced in the absence of T cells, dark zones form without the requirement for T cell selection (Vinuesa et al., 2000). Presumably because antigen available for selection is absent, the B cells in both the light zones and dark zones of these GC undergo involution by mass B cell apoptosis within 5 days of GC induction. If dark zone B cells are not moving to the light zone where they are selected, why should this involution occur in such GC? Hypotheses of the way affinity maturation occurs in GC set out many years ago have been widely accepted, but are not necessarily adequately tested. We must be grateful to Wang and Carter (2005) for reminding us of the uncertainty that exists about the way B cells are selected during affinity maturation in GC.

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Infection, Autoimmunity, and Glycolipids: T Cells Detect Microbes through Self-Recognition

De Libero et al. (2005) demonstrate in this issue of *Immunity* that bacterial infection leads to increased synthesis of autologous glycolipids that are recognized by CD1-restricted human T cells, indicating that recognition of inducible self-glycolipids could be a mechanism for microbial detection. This mechanism also may provide a connection between infection and autoimmunity.

CD1 molecules present glycolipids to T cells, but we are just coming to understand the full physiologic significance of glycolipid recognition. CD1 molecules are nonpolymorphic antigen-presenting molecules that are categorized by sequence similarity into two groups (Porcelli, 1995). The group I CD1 molecules include human CD1a, CD1b, and CD1c and their orthologs in other species, whereas the more distantly related CD1d molecules constitute group II. Mice and rats are unusual among mammals in only having group II CD1d molecules. Human group I CD1 molecules have been shown to present lipid antigens from the cell wall of mycobacteria, and the reactive T cells have diverse TCRs and produce Th1 cytokines (Brigl and Brenner, 2004). Therefore, lipid antigen recognition in this way apparently is a useful branch of T cell adaptive immunity. By contrast, many of the T cells that recognize antigens presented by CD1d have an invariant Vα14 (Vα14i) TCR in mice, and these cells, sometimes called Vα14i natural killer T (NKT) cells or iNKT cells, are autoreactive for the glycosphingolipid isoglobotrihexosylceramide (iGb3) presented by CD1d (Zhou et al., 2004). The homologous human iNKT cells, with an invariant Vα24 (Vα24i) rearrangement, have a similar specificity. These facts suggested that there may be a functional dichotomy between the group I and group II CD1 molecules that correlates with sequence divergence. According to this view, the group I molecules are important for adaptive immunity and group II molecules are important for self-recognition that might somehow be beneficial for immune regulation, by analogy with the self-reactive $CD4^+$ $CD25^+$ regulatory T cells.

The results from several experiments, including the work by De Libero and colleagues (De Libero et al., 2005), suggest that this simple dichotomy may not be correct. Instead, both group I and group II CD1 molecules may be important for detecting microbial infections by direct recognition of foreign antigens or inducible self-antigens (Figure 1). For example, iNKT cells

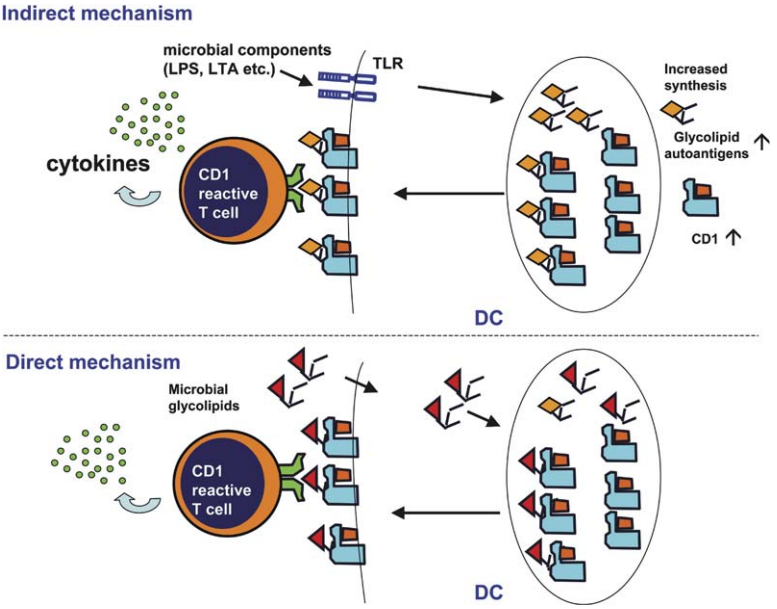

Figure 1. CD1 Reactive T Cells Detect Microbial Infection by Recognition of Inducible Autoantigens or Microbial Antigens

Bacterial components such as lipopolysaccharide (LPS) or lipoteichoic acid (LTA) increase the synthesis of glycolipid autoantigens, resulting in the activation of CD1-reactive T cells (indirect mechanism). In the other pathway, CD1 molecules activate T cells by presenting microbial antigens to these cells (direct mechanism). The diagram illustrates glycolipids rather than bacteria being taken up by the DCs, although this is not necessarily the case.

recently were shown to react to glycosphingolipids from *Sphingomonas* bacteria presented by CD1d (Kinjo et al., 2005; Mattner et al., 2005). Therefore, the same TCR that reacts to the autologous glycosphingolipid iGb3 also reacts to bacterial glycolipids.

Surprisingly, the self-reactivity of Vα14i NKT cells for CD1d$^+$ dendritic cells (DCs) also is connected to microbial stimulation, because exposure of DCs to bacterial products stimulated Vα14i NKT cell cytokine release (Brigl et al., 2003; Mattner et al., 2005). This Vα14i NKT cell response required IL-12 secretion by the stimulated DCs (Brigl et al., 2003), but it also required CD1d expression and the enzyme that generates iGb3 (Mattner et al., 2005), indicating TCR-mediated autoreactivity. Therefore, the self-reactivity of Vα14i NKT cells can serve as an indirect mechanism for the detection of bacteria.

Regarding the group I CD1 molecules, De Libero and colleagues showed previously that they are not exclusively involved in microbial antigen presentation (Shamshiev et al., 1999). They characterized, from peripheral blood, autoreactive T cells that respond to glycosphingolipids such as sulfatide and ganglioside GM1, which are abundant in the central nervous system. The frequency of these autoreactive cells was increased in the peripheral blood of multiple sclerosis (MS) patients (Shamshiev et al., 1999), and modulating sulfatide autoreactivity was shown to be important for the pathogenesis of experimental autoimmune encephalomyelitis, an animal model of MS (Jahng et al., 2004).

In the current study (De Libero et al., 2005), De Libero and colleagues have asked if microbial infections could contribute to glycosphingolipid autoreactivity and autoimmunity. They demonstrated that exposure of DCs to bacteria or bacterial products stimulated different T cell clones autoreactive for sulfatide presented by either CD1a or CD1b, or for ganglioside GM1 presented by CD1b. Whereas this exposure caused increased expression of CD1 and B7 costimulatory molecules by the APCs, this was not sufficient for the increased T cell responsiveness, as Lipid A could cause a similar increase in surface-molecule expression without stimulating autoreactivity. Fixation before exposure to bacterial products prevented the increased autoreactivity, indicating that an active metabolic process in the APCs was required. The results from two experiments demonstrated that this was likely to be due to increased synthesis of the glycosphingolipid autoantigens. First, a biochemical analysis indicated increased levels of the glycolipids in cells exposed to bacteria or LPS. Second, treatment of APCs with an inhibitor that blocks an early step in glycosphingolipid biosynthesis led to decreased LPS-induced autoreactivity without affecting the ability to recognize exogenously added sulfatide or GM1.

Collectively, these results suggest that the CD1 group I and group II reactive T cells constitute a subset of lymphocytes adapted to respond to microbes, either through direct recognition of their glycolipids or indirectly, through the recognition of microbe-induced autologous antigens, many of which are glycosphingolipids (Figure 1). Autoreactivity has a potentially detrimental side, however, as it can lead to autoimmunity if not properly regulated. Microbes may contribute to autoimmunity through molecular mimicry, which is crossreactivity between microbial and self-antigens. The manuscript in this issue suggests a second type of antigen-specific connection between infection and autoimmunity, namely the induction of the synthesis of particular glycolipid autoantigens.

Broad generalizations, such as the scheme outlined in Figure 1, are inevitably dangerous, however, and it remains to be determined if iGb3 synthesis also is induced by microbial stimulation and if the CD1 group I reactive T cells reactive to self and foreign antigens are overlapping, as for the group II CD1-reactive T cells. Moreover, the two CD1 systems differ in several as-

pects, including the absence of a TCR invariant population reactive to the group I CD1 molecules and possibly also with regard to the requirement for IL-12 synthesis to stimulate group I CD1 autoreactivity. Regardless, the overall similarities are striking, and they suggest the evolution of a glycolipid antigen recognition system with unique capabilities for the detection of microbial organisms.

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